

Attorney Docket No.: UT-0006  
Inventors: Rao et al.  
Serial No.: 09/109,858  
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Please amend the claims as follows:

12. (Thrice amended) A method of isolating a pure population of rodent or human CNS neuron-restricted precursor cells comprising the steps of:
- (a) isolating a population of rodent or human multipotent CNS stem cells which generate both neurons and glia;
  - (b) incubating the multipotent CNS stem cells in NEP medium;
  - (c) replating the multipotent CNS stem cells on laminin in the absence of chick embryo extract to induce cell differentiation;
  - (d) purifying from the differentiating cells a subpopulation of cells expressing embryonic neural cell adhesion molecules via a procedure selected from the group consisting of specific antibody capture, fluorescence activated cell sorting, and magnetic bead capture; and
  - (e) isolating a pure population of rodent or human CNS neuron-restricted precursor cells via incubating the purified subpopulation of cells in a FGF-containing medium configured for supporting adherent growth thereof wherein said isolated pure population of neuron-restricted precursor cells differentiates into CNS neuronal cells upon replacement of adherent growth supporting

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medium with retinoic acid containing medium and wherein said isolated pure population of neuron-restricted precursor cells fails to proliferate or differentiate into CNS glial cells in astrocyte-promoting medium containing FGF and 10% fetal calf serum.

21. (Thrice amended) A method of isolating a pure population of rodent or human CNS neuron-restricted precursor cells comprising the steps of:

(a) removing a sample of spinal cord tissue from a rodent or human embryo at a stage of embryonic development after closure of the neural tube but prior to differentiation of glial and neuronal cells in the neural tube;

(b) dissociating cells comprising the sample of spinal cord tissue removed from the embryo;

(c) purifying from the dissociated cells a subpopulation expressing embryonic neural cell adhesion molecule;

(d) plating the purified subpopulation of cells in feeder-cell-independent culture on a substratum and in a medium configured for supporting adherent growth of the neuron-restricted precursor cells; and

(e) isolating a pure population of rodent or human CNS neuron-restricted precursor cells via incubating the plated cells at a temperature and in an atmosphere conducive to growth wherein

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said isolated pure population of neuron-restricted precursor cells requires FGF for adherent growth, differentiates into CNS neuronal cells upon replacement of adherent growth supporting medium with retinoic acid containing medium and fails to proliferate or differentiate into CNS glial cells in astrocyte-promoting medium containing FGF and 10% fetal calf serum.

59. (Twice amended) A method of isolating a pure population of mouse CNS neuron-restricted precursor cells comprising the steps

- of:
- (a) providing a sample of mouse embryonic stem cells;
  - (b) purifying from the mouse embryonic stem cells a subpopulation expressing embryonic neural cell adhesion molecule;
  - (c) plating the purified subpopulation of cells in feeder-cell-independent culture on a substratum and in a medium configured for supporting adherent growth of the neuron-restricted precursor cells; and
  - (d) isolating a pure population of mouse CNS neuron-restricted precursor cells via incubating the plated cells at a temperature and in an atmosphere conducive to growth of the neuron-restricted precursor cells wherein said isolated pure population of neuron-